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THE ACTION OF POTASSIUM ON THE SUPERIOR CERVICAL GANGLION OF THE CAT.

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In searching for a non-specific stimulant of ganglion cells, Feldberg and Vartiainen [1934] found that KCl injected into the perfused superior cervical ganglion of the cat had a number of actions, viz. at a certain dosage the ganglion was excited to discharge, doses in excess of this paralysed conduction through the ganglion, whereas doses in themselves insufficient either to excite or to paralyse produced a potentiation of the effects of a series of submaximal preganglionic stimuli.

In the experiments here recorded, we have examined the effects of K^+ on the response of the perfused superior cervical ganglion of the cat to single preganglionic volleys. The excitant effects of K^+, which we observed, led us to investigate whether these and other ions liberated acetylcholine (ACh.) from the ganglion. The third section of this paper is concerned with the paralysing actions of K^+.

It seemed to us of primary importance to determine what effect, if any, K^+ had upon the conduction through the ganglion of single preganglionic volleys, since the potentiating effect produced by a substance might be due to one or more of four possible processes: (*a*) the lowering of ganglion-cell threshold, so that previously subthreshold stimuli become effective; (*b*) an alteration in ganglion-cell refractory period and changes in the facilitation process involving rapidly succeeding excitations of the ganglion cell; (*c*) the initiation of after discharge of the ganglion; and (*d*) alterations in the amount or the distribution, temporal or spatial, of a transmitting substance.

A study of the relation existing between the K^+ content of the perfusion fluid and the appearance of ACh. in the venous effluent of the ganglion appeared the more important in view of the known intimate

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relationship between the nervous impulse and K ions. That the propagation of a nervous impulse is, in crabs' peripheral nerves, associated with a mobilization of K^+ has been established by Cowan [1934]. More relevant to the present experiments are the recent findings of Vogt [1936] that the superior cervical ganglion certainly loses K^+ during excitation of the preganglionic cervical trunk in the dog. In addition to this there is already some warrant for the assumption of a relationship between K^+ and ACh. in peripheral nervous structures; Beznák [1934] has stated that increase in the K^+ concentration of the fluid perfusing a frog's heart leads to the appearance of ACh. in the perfusion fluid, a result which may have a connection with the old observation of Howell and Duke [1908] that vagus stimulation increases the K content of the fluid perfusing a dog's heart. It is not unreasonable to suppose that such a concomitant of the nerve impulse as a liberation or mobilization of K ions might be the agent responsible for the discharge of ACh. at the terminations of the nerve. On this supposition it could be readily conceived that a sudden artificial disturbance of the ionic balance in a perfused ganglion might occasion a discharge of ACh.

Methods.

Ganglion perfusion was carried out in the manner previously described [Feldberg and Gaddum, 1934; Feldberg and Vartiainen, 1934]. The administration of excess KCl was usually effected by injecting a small volume of warmed fluid through the rubber tubing at the nose of the cannula, but, in those experiments in which a more prolonged exposure of the ganglion to a solution rich in K^+ was desired, two perfusion systems and two cannulæ were used. One cannula was tied into the common carotid artery and the other in the external maxillary artery. The change of solution was then conveniently effected by closing off the tube to one cannula, washing out the dead space with the fresh solution, and then closing the appropriate artery with a clamp applied as closely as possible to the ganglion. In those experiments in which the liberation of ACh. in the ganglion was investigated, eserine was added to the perfusion fluid to make a concentration of 1 in 5×10^5.

The conduction of single preganglionic volleys and pairs of volleys was first investigated by recording ganglionic action potentials. The uncertainty of the effects of K^+ on the action potentials led us to attempt further analysis by recording the responsive changes in the isometric tension of the nictitating membrane.

Action potentials. The postganglionic bundle was tied and cut as far as possible distal to the ganglion. In a number of experiments, the ganglion together with its vessels was removed from the animal as soon as perfusion had been initiated, and the experiment was made with the nerve, vessels and ganglion preserved in a warmed vulcanite trough. More usually, after perfusion had been started, the animal was removed to a warmed, earthed metal box, the inside of which was insulated with sheet rubber. It was, of course, necessary to screen not only the preparation but also the whole perfusion apparatus. All tubes conducting the perfusion fluid were carried in earthed flexible metal gas tubing, and the perfusion fluid, heating circuit and distance thermometer were also screened in a metal box, connected by metal tubes to the screening box for the cat. This large extent of earthed metal tubes, connected so intimately with the preparation from which the records are taken, introduces capacity effects which render stimulus escape a serious problem, and the picking up of artefacts from surrounding electrical machinery is also a considerable annoyance.

The lead-off electrodes, Ag-AgCl-Ringer, with yarn wicks, were applied, the grid lead to the crushed end of the postganglionic nerve and the earthed lead to the middle of the ganglion. A resistance-capacity coupled amplifier of the usual type was used, the coupling condensers being of such size as to give a deflection falling to half its original value in 0·52 sec. when a rectangular current was applied to the input (Fig. 1 A). The stimulating electrodes were usually of the same type as those used for leading off, since they give less trouble with stimulus escape and the drying of the nerve is less than with simple silver chloride coated wires.

The stimuli were delivered from two induction coils, placed far apart and at right angles. The primary and secondary coils were loosely coupled and 12 volts reduced by a suitable resistance provided the primary current. Break shocks timed by a Lucas pendulum were used. Condenser stimulation was abandoned on account of the severe escape produced. The KCl solutions were injected in amounts between 0·1 and 1·0 c.c. In order to facilitate the immediate observation of the effects of the injection, the syringe was actuated by a Bowden cable which passed outside the screening box and enabled the injection to be made without upsetting the amplifier. Care was taken to warm the injected solution and, by adjustment of the heating current, to maintain the temperature of the perfusion as nearly constant as possible.

In those experiments in which the response of the nictitating membrane to single and double preganglionic volleys was recorded, we

used the frictionless torsion myograph as previously described [Brown, 1934].

Ganglion extracts. In the course of the experiments it was found necessary to estimate the ACh. content of ganglia. The ganglia were cleanly excised from the anæsthetized animal, freed from connective tissue and wiped dry with filter paper; after rapid weighing they were transferred to a small vessel containing 1 c.c. of 10 p.c. trichloroacetic

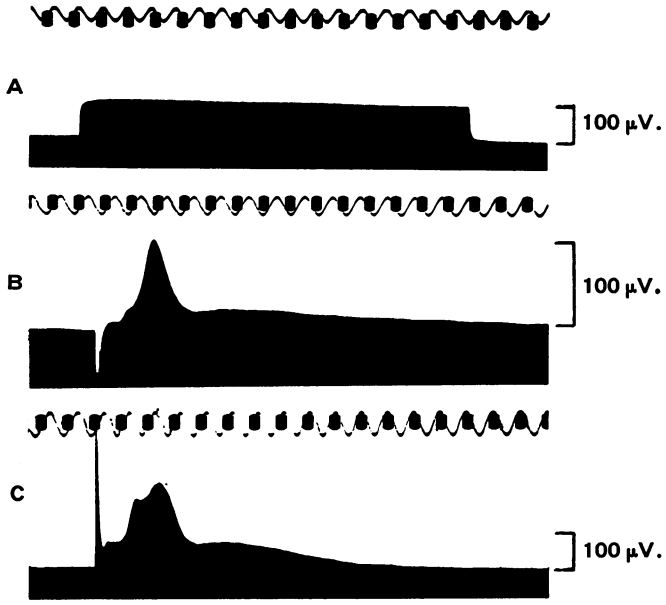

Fig. 1. A. Response of amplifier to rectangular potential applied to input. B. Action potential of ganglion after 5 hours' perfusion. C. Action potential of ganglion with intact blood supply. Time 10 msec. The initial deflection, of opposite sign in B and C, is the stimulus artefact.

acid per ganglion and there minced very finely with scissors. The acid was allowed to act for 1 hour with occasional shaking, and the suspension was then filtered through filter paper. The filtrate was shaken a number of times with ether to remove the acid, the reaction was adjusted to about *p*H 4 with HCl and the filtrate was then dried at low pressure and at a temperature not exceeding 40° C. The perfectly dry residue was taken up with dry alcohol, the *p*H adjusted and the solution again dried; the residue was dissolved in a small volume of distilled water and its ACh. content estimated on the frog's rectus, the leech muscle preparation or the cat's blood-pressure.

Assay of ACh. As a test object for the ACh. content of solutions containing excess KCl we used either the dorsal body-wall muscle of the leech, sensitized with eserine, or the blood-pressure of the cat. It is only possible to use the leech when the fluid tested contains small concentrations of K^+, and, in any event, this must be adjusted by dilution with a K-free solution to a concentration not higher than that in normal Locke's solution; otherwise, undesirable contractions of the leech are produced by the K^+ itself, which interfere with the accuracy of the assay. In those experiments in which higher concentrations of K^+ were used than were conveniently brought within physiological limits by dilution for testing on the leech, the cat's blood-pressure, after special preparation of the animal, provided almost as sensitive and satisfactory a test as the leech. The cats were anæsthetized with chloralose (0·08 g. per kg.) early in the day and were left untouched for from 2 to 4 hours. Immediately before the test was made the circulation was restricted by removal of the abdominal viscera, and eserine (0·1–0·15 mg. per kg.) was administered intravenously. With these precautions a good fall of blood-pressure may, in exceptional animals, be produced by as little as 0·001γ of ACh., while a reasonable sensitivity to 0·005γ of ACh. is common. A disadvantage of the preparation is that the sensitivity of the animal to ACh. decays fairly rapidly; and, although it is sensitive to small amounts of ACh., its discrimination between different doses is often relatively poor. The identity with ACh. of the substance appearing in the venous effluent was established by the disappearance of the depressor action on the cat after atropine in small doses, by its destruction in weak alkali at room temperature, and by its failure to appear when eserine was omitted from the perfusion fluid of the ganglion.

Experimental.

(1) *The effect of potassium on transmission of single preganglionic volleys.*

A. *Action potentials of perfused ganglia.*

The perfused ganglion excited by single preganglionic volleys produces an action potential which shows, in general, all the features of the action potential of the ganglion with natural circulation. The actual value of the E.M.F. produced is, however, invariably much less in the perfused ganglion, values of about 150μV. being usual as compared with 500μV. in the normal ganglion. There is, moreover, a progressive falling off of the E.M.F. produced as the experiment proceeds. This reduction in action potential appears to be due largely to the short-circuiting of the input

by oozing Locke's solution from the perfusion, and also to the gradually developing and inevitable waterlogging of the connective tissue sheath of the ganglion. That the reduced action potential is not a result of the failure in part of the ganglion to respond, is evident from the fact that the response of the nictitating membrane to single preganglionic volleys compares very favourably with that occurring in animals in which the ganglion has not only a natural circulation, but has not even been exposed. Fig. 1 B shows the action potential of a ganglion after 5 hours' perfusion, compared with that from a ganglion with natural circulation (C).

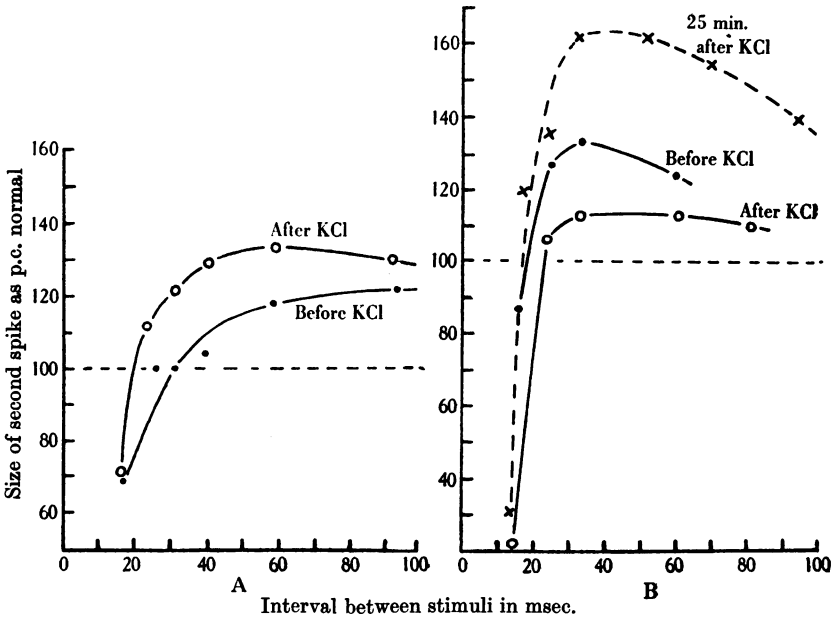

Fig. 2. Effects of KCl injection on facilitation between two succeeding volleys.

The compound spike is indicative of fibres of varying conduction velocity. It is noteworthy that the perfused ganglion shows, in addition to the spikes, the slow negative and positive variations which Eccles [1935 *c*] associates with the processes of facilitation and inhibition.

A few determinations of the absolute refractory period of the ganglion have been made which indicate that the refractory period of the fastest fibres of the perfused ganglion is somewhat increased, viz. 5–6 msec. as against 2·9–4·7 msec. in the normal ganglion. The perfused ganglion shows the processes of facilitation described by Eccles in the ganglion with intact blood supply. Fig. 2 shows the time relations and extent of the facilitation between two preganglionic volleys.

Effects of potassium. The effects of injected KCl solution are small and transient unless high concentrations are given. In the latter instance, the effect is always the complete paralysis of the ganglion and the abolition of all action potentials. With small doses, *i.e.* 0·1–0·8 c.c. of a solution containing 1·4 mg. per c.c. KCl, an increase in the magnitude of the spike potential is observed. The largest increase we have seen is 25 p.c. over the original size. This increase of spike height is only seen when the exciting stimuli are very submaximal. Maximal ganglionic potentials are never increased by KCl injection. The response of the ganglion to stimuli which are nearly maximal gives a clear demonstration of this failure to augment the maximal spike; with certain strengths of stimuli, KCl injection may cause an increase in the spike height of the slower waves, whereas the fast waves, due to more excitable fibres which are presumably maximally stimulated, remain unchanged. A few determinations of the refractory period have been made after KCl injection, but no significant alterations have been detected.

We have further studied the effect of KCl on the facilitation between two volleys. Both increase (Fig. 2 A) and decrease (Fig. 2 B) have been observed, and we have been unable to detect any constant change. One fact which renders difficult the interpretation of such effects is that any increase in spike height produced by KCl will bring about, *pari passu*, a diminution in the percentage increase in the second of two volleys, since the facilitation process depends on the bringing into activity of the subliminal fringe of a submaximally excited ganglion. The action of KCl in increasing the spike of a submaximal excitation must diminish the available increment of subliminally excited cells and consequently reduce the measurable facilitation. Fig. 2 shows facilitation curves obtained before and after an injection. The danger of attaching importance to small changes in the facilitation curve is shown by the fact that an injection of Locke's solution into the perfusion fluid produced, probably by temperature change, a diminution in the height of the first spike and a consequent apparent increase in facilitation.

B. *Response of the nictitating membrane.*

Single volleys set up in the preganglionic cervical sympathetic produce, when passing through the perfused ganglion, as in the ganglion with intact circulation, single "twitches" of the nictitating membrane. Maximal single stimuli may produce tensions of 1·8 g., which is little different from the tensions developed when the ganglion has its natural circulation (2 g.).

The main features of the action of KCl conform very closely with those obtained with the action-potential records. The effects are best seen when two stimuli separated by some 20–100 msec. are applied to the preganglionic fibres, provided that the stimuli be submaximal. In these circumstances, increases in tension up to 100 p.c were observed following injection varying between 5 c.c. of 0·09 p.c. KCl and 0·2 c.c. of 0·14 p.c. KCl. Similar but smaller increases in tension in response to single volleys were observed. Here again, as with the action potential, it is essential that the stimulus be reduced to a low value, stimuli at just above the threshold intensity, or about one-fifth maximal, giving the best opportunity for showing the KCl potentiation. With single volleys, the greatest increase in tension observed was 60 p.c. In a few experiments we have determined the summation curve with maximal preganglionic volleys in the manner previously described [Brown, 1934]. No significant alterations were produced by KCl injection.

(2) *Liberation of acetylcholine by ionic changes.*

A. *Experiments on normally innervated ganglia.*

The venous effluent at the beginning of the perfusion of a ganglion with an eserinized Locke solution often contains some ACh., the concentration being rarely higher than 0·01γ per c.c. The concentration decreases regularly, and ACh. has usually disappeared after less than 30 min. of perfusion. The use of a solution containing less KCl than the usual concentration does not lessen this "spontaneous" initial output. We have accordingly allowed the perfusion to proceed until the venous outflow was found to contain not more than insignificant traces of ACh., before any injections were made. More recent experiments have shown that the "spontaneous" output is probably due to the persistent injury discharge of the cut nerve, since it is absent if the nerve is divided some two hours before the beginning of the perfusion.

Potassium and sodium. The effect of a sudden increase in the K^+ concentration of the perfusion fluid is the appearance of considerable quantities of ACh. in the venous outflow. Just detectable amounts are already evident, when the K^+ concentration of the injected fluid is raised to twice the normal. The amount of ACh. increases as higher concentrations of KCl are used, until after an injection of 2–5 mg. of KCl in 0·5 c.c. of fluid, the next c.c. of venous outflow contains 0·02–0·1γ of ACh. An additional action of KCl is the production of an intense vaso-constriction in the ganglion, which can in part be overcome by raising the perfusion pressure.

It is evident that such large increases in the salt concentration of the perfused fluid must induce a considerable osmotic action on the ganglion, but we were able to establish that the effect of KCl was not due to this osmotic change. Injections of the same amounts of NaCl have no, or at the best a very slight effect. Fig. 3, for instance, shows the assay on the cat's blood-pressure of the venous effluent after the injection of 5 mg. of NaCl (A) and of 5 mg. of KCl (B). It will be seen that the NaCl venous outflow has only a slight depressor effect, compared with the pronounced effect of the KCl sample. So slight and inconstant has the NaCl effect been, that we have made no attempt to examine it in detail. In order, however, to exclude any osmotic component in the effect of KCl, we have so reduced the NaCl in the injected fluid as to make an isotonic solution.

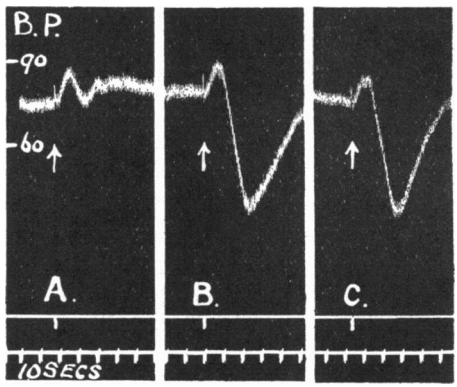

Fig. 3. Cat chloralose, blood-pressure. Effect of 1 c.c. venous effluent from ganglion. A, after 5 mg. NaCl, B, after 5 mg. KCl and C, ACh. 0·025γ in 1 c.c.

Another action of the injected KCl was stimulation of the ganglion, as evidenced by the retraction of the nictitating membrane, followed, if higher amounts of KCl were injected, by a quick relaxation, due to the secondary, paralysing action of potassium. The threshold for the stimulating effect of KCl is very near to, if not identical with, the threshold for the appearance of ACh. in the venous outflow, but, as will be shown, the stimulating effect of KCl cannot be attributed wholly to the ACh. which it liberates.

In addition to injecting small volumes of a solution with a high K^+ content, we have also studied the effects of more prolonged perfusion of the ganglion with solutions rich in potassium. Change of the normal perfusion fluid for one containing four times the concentration of KCl leads to an output of ACh. which continues, with gradual diminution, for

20–60 min. Prolonged perfusion with a solution rich in KCl, after a preliminary stimulation, paralyses the ganglion to preganglionic impulses and to ACh. If the solution now be changed for the normal, complete recovery of the ganglion ensues and a subsequent return to a solution of high KCl concentration again discharges ACh.

Calcium. $CaCl_2$ does not liberate ACh.; it has, in fact, the effect of inhibiting the action of excess K^+. In one experiment, for instance, 5 mg. $CaCl_2$ failed to discharge ACh., whereas KCl in the like amount

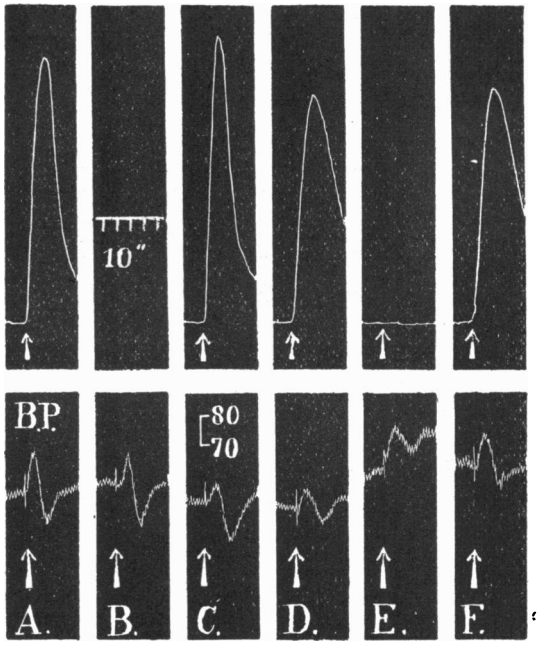

Fig. 4. Contraction of nictitating membrane following injections into perfused ganglion of K^+ and Ca^+. Cat blood-pressure—effect of venous effluent collected after corresponding injections. B, 0·01γ ACh. For details see text.

caused the output of ACh. in a concentration of 1 in 3×10^7. In Fig. 4 a series of injections into the ganglion perfusion are shown, each of 0·5 c.c. of solution containing a variety of multiples of the normal K^+ and Ca^+ concentrations. At C, injection KCl 8 × normal caused an output of more than 0·01γ ACh. The reduction in ACh. output following the addition of $CaCl_2$ 4·5 × and 8 × the normal to the KCl solution is shown at A and D respectively. The injection of KCl × 8 and $CaCl_2$ × 16 was followed by no output whatsoever (E). The persistence of the $CaCl_2$ depression is shown in the reduced effect of injection of KCl alone at F,

13 mins. afterwards, as compared with that of C. It is clear from this that although there exists a demonstrable antagonism between K^+ and Ca^+ the preponderance of activity is with the K^+. It may be noted that the injection of a Ca^+ free solution containing the normal concentration of K^+ was without any detectable effect.

$CaCl_2$ has no stimulating action on the ganglion cell and, if given together with KCl, it lessens and, in sufficient concentration, abolishes the excitant effect of the KCl (Fig. 4 E). $CaCl_2$ causes vaso-dilatation in the ganglion.

Cæsium. CsCl has only a very weak effect in liberating ACh., even if injected in amounts of 20 mg. per c.c. It lacks also the intense vaso-constricting action described for KCl. On the other hand, it stimulates the ganglion cells, as shown by the retraction of the nictitating membrane. This stimulating action is much more prolonged than that caused by KCl and does not seem to be followed by a paralysing effect.

Rubidium. RbCl causes an output of ACh. from the ganglion, though the amount liberated seems to be somewhat smaller than that liberated by equal doses of KCl. It causes further an intense vaso-constriction and stimulation of the ganglion cells.

B. *Experiments on denervated ganglion.*

There is evidence associating the presence in an organ of a specific chemical transmitter with the integrity of the appropriate nerve fibres [for literature see Gaddum, 1936], and the data available point to a preganglionic origin of the ACh. in the superior cervical ganglion [Feldberg and Vartiainen, 1934]. The KCl injection technique provides a very suitable method for examining, in a denervated ganglion, in which preganglionic stimulation is impossible, not only the excitant action of KCl, but also the relation between the integrity of the preganglionic fibres and the ACh. output of a stimulated ganglion.

The cervical sympathetic was divided aseptically under ether anæsthesia in a number of cats. After an interval of between 3 and 6 weeks the denervated ganglion was perfused in the usual way and KCl injected. In a denervated ganglion such injections produce either no detectable ACh. or amounts which are only just detectable (0·001–0·0015γ). This result is strikingly different from that observed in the normal ganglion; it is explicable on the assumption, either that the ACh. of the ganglion is so closely related to the preganglionic fibres that it disappears when these degenerate, or that the denervation so changes the ganglion that ACh. cannot now be liberated when KCl is injected.

Estimation of the ACh. equivalent of extracts of ganglia, innervated and denervated, gave an answer to this question. Innervated ganglia yielded the high ACh. equivalent 10–20γ per g. The variation between right and left normal ganglia did not exceed 15 p.c. After denervation the ACh. content of the ganglia fell to 1–3γ per g. Table I gives the ACh.

TABLE I.

Number of ganglia extracted together	ACh. equivalent in γ per g. fresh weight		Method of assay
	Normal	Denervated	
3	16	3	Frog rectus
1	19	2	Cat blood-pressure
1	10	1	Cat blood-pressure
2	13	2	Leech
1	12	2	Cat blood-pressure
Mean	14	2	

equivalents of the extracts of normal and denervated ganglia. This result shows clearly that the high ACh. content of the normally innervated ganglion is dependent upon the integrity of the preganglionic fibres, and elucidates the failure of KCl to liberate ACh. in significant amounts from the denervated ganglion.

The close association between the appearance of ACh. and recordable excitation of the ganglion cells in response to KCl, naturally led us to suspect that the stimulating action of KCl might be due, not to the ion directly, but to the liberated ACh. We found, however, that the denervated ganglion, in which relatively little ACh. is available for discharge, is not measurably less excitable than the normal ganglion by KCl. It was not feasible to compare exactly the threshold and response of the normal and denervated ganglia, since preganglionic denervation *per se* alters the characteristics of the nictitating response [Hampel, 1935], quite apart from any effect which it may have upon the ganglion. Individual ganglia moreover differ in their responsiveness to KCl, to a degree greater than any differences we were able to detect between normal and denervated ganglia.

(3) *The paralysing action of* KCl.

We have already referred briefly to the paralysing effect of short exposure to high concentrations of KCl or prolonged perfusion with lower concentrations.

The paralysing effects of KCl may be contrasted very strongly with its persistent action in discharging ACh., when, instead of injecting the excess salt, the perfusion fluid is changed entirely for one containing

a high K^+ concentration. In these circumstances a small and relatively transient contraction of the nictitating membrane occurs, whereas, as shown previously, a long lasting output of ACh. ensues. During this period there is no response to preganglionic stimulation, although this still liberates additional amounts of ACh. At this stage of the paralysis, the liberating mechanism is as yet unaffected, whereas the cell is paralysed to the effect of ACh. The paralysis produced is analogous to that produced by nicotine, eserine [Feldberg and Vartiainen, 1934] and curarine [Brown and Feldberg, 1936].

If perfusion is further continued a condition is reached in which preganglionic stimulation is ineffective in liberating ACh. If, when a ganglion has reached this stage, the perfusion fluid is changed for one containing the normal KCl concentration, recovery ensues in an exactly

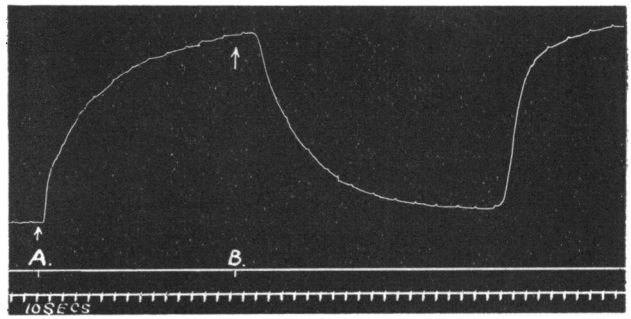

Fig. 5. Contraction of nictitating membrane. A, maximal preganglionic stimulation from A to end of record. B, injection into ganglion of 2·5 mg. KCl in 0·5 c.c.

reverse order. At first stimulation produces no ACh. and no effect on the nictitating membrane; then, after a passage of some 30 min., renewal of stimulation causes ACh. to appear but no contraction of the nictitating membrane occurs; and finally, after about 50 min., stimulation of the nerve causes contraction of the nictitating membrane and output of ACh. as normally.

Although KCl manifests a potent paralysing effect on ganglion cells, this paralysing action does not appear to extend to the fine terminal filaments of the preganglionic fibres until long after the ganglion cells have been seriously affected. This may be conveniently demonstrated if such a frequency for preganglionic stimulation is chosen as to cause a prolonged and maintained contraction of the nictitating membrane. If during such a stimulation a small volume of KCl solution is injected, there ensues, after a latency which varies with the velocity of the per-

fusion flow, a complete inhibition of the contraction (Fig. 5). Nevertheless, throughout the continued stimulation, the output of ACh. continues at a level as high as during the full contraction of the nictitating membrane. If the stimuli are sufficiently submaximal, the inhibition is preceded and followed by a small but distinct augmentation of the contraction.

$CaCl_2$ also has the property, when injected during a prolonged stimulation, of causing inhibition, the cell being also paralysed to injected ACh. The inhibitory effect of $CaCl_2$ on the stimulant actions of KCl has already been considered (Fig. 4 A, D, E).

Discussion.

The experiments here reported show that the physiological responses of the perfused ganglion are little different from those of the normal tissue, a finding which is not surprising in view of Eccles' [1935 *b*] statement that interruption of the blood supply does not seriously impair ganglionic conduction, provided the temperature is maintained.

We have shown that excess of K ions in the ganglion increases both the action potential and the tension evoked in the nictitating membrane by single stimuli, provided that these preganglionic stimuli are sufficiently weak to fail to excite the nerve maximally. One explanation consistent with the experimental facts is that K ions act by so lowering the cellular threshold that cells previously not excited now discharge. Alterations in refractory period of ganglion cells and alterations in the transmission of succeeding impulses might explain the potentiation which is observed with a *series* of submaximal preganglionic volleys, but this does not apply to the effects of single volleys. That the initiation of after discharge cannot be invoked in explanation, is demonstrated by the failure of K to increase the effects of single *maximal* volleys. In this connection attention may be drawn to the inadequacy of the electrical record of the ganglionic activity as a measure or index of after discharge. The large potentials developed by the ganglion on stimulation make the detection of the firing of small fibre groups impossible, more especially since any such discharge is almost certain to be asynchronous.

As we have pointed out in the introduction to these experiments, Vogt's [1936] work gives us some reason to assume that excitation of the ganglion cell originates a diffusion outwards of K ions. Feldberg and Vartiainen [1934] have already suggested that the K ion may be responsible for the persistent excitatory condition of the cell following the transmission of an impulse. Our finding that an artificially induced increase in the K ion concentration around the cell may give rise to a

persistent subliminal excitement gives some support to this supposition. Eccles [1935 *d*], in a recent communication, has found that even antidromic excitation of the ganglion may produce a persistent subthreshold excitatory state of the cell, although inhibition predominates, a further reason, in our opinion, for associating the similar effects persisting after a preganglionic volley with ionic changes in, or around, the cell.

Although we have no direct experimental proof that the excitant effects of KCl are entirely dissociated from the ACh. which it liberates, the persistence, apparently unchanged, of the stimulant action of KCl on the denervated ganglion, in which only insignificant amounts of ACh. are liberated, proves that the injected KCl has of itself a strong stimulant action on the cell. The stimulation has closely associated with it a paralysis of the cell to ACh., and this paralysis may easily interfere with the stimulating action of the ACh. liberated by the K ion. This may explain the similarity of the responses of the normal and the denervated ganglion to KCl.

We have now evidence to associate not only ACh. but also K ions with many of the phenomena of conduction in the superior cervical ganglion. Eccles [1935 *a*] has suggested that the ACh. liberated in a ganglion by preganglionic stimulation plays only a subsidiary role, and that the actual transmission is by the K ions conveying "eddy currents" set up by the preganglionic impulses. We have been able recently [Brown and Feldberg, 1936] to produce direct evidence, which appears to exclude such a predominant and direct function of K ions in the actual transmission of the impulse across the synapse. Curarine, in suitable doses, was found to paralyse the ganglion cell to preganglionic stimulation and to ACh. while leaving the cellular response to KCl actually enhanced. During curarine paralysis the ACh. output from the preganglionic terminals is unaffected. The possibility is not excluded that the function of ACh. as the specific transmitter, like its liberation, may be conditioned by the appearance of K ions at the synapse; but the evidence is against any conception of the K ion as the essential transmitter.

The liberation of ACh. by K ions, as established in these experiments, and the probable association of the K ion with the propagated disturbance in nerve, make the assumption not unreasonable that the wave of mobilized K ions accompanying the nerve impulse liberates ACh. at the nerve terminals. This ACh. may then be pictured as stimulating the ganglion cell to discharge, thus starting, as a separate event, the postganglionic impulse, which may again be a wave of mobilization of K ions.

SUMMARY.

1. Action-potential records of the perfused superior cervical ganglion of the cat show that conduction under these conditions differs little from that in the ganglion with normal blood supply.

2. Small doses of potassium chloride injected into the perfusion fluid increase the response of the ganglion to single submaximal preganglionic volleys.

3. Potassium ions liberate acetylcholine from the normally innervated ganglion. A similar property is possessed by rubidium and to a very weak degree by cæsium. Sodium and calcium have not this action. Calcium inhibits the liberation of acetylcholine by potassium ions.

4. The normal ganglion contains 10–20γ per g. of acetylcholine. After degeneration of the preganglionic fibres the acetylcholine falls to 1–3γ per g. The fall explains the fact that potassium ions liberate only insignificant amounts of acetylcholine from such a denervated ganglion.

5. Potassium stimulates the cells of both normal and denervated ganglia to discharge.

6. In large doses, potassium has a paralysing effect on ganglion cells; this paralysis does not extend at first to the terminal filaments of the preganglionic fibres, acetylcholine being still liberated by preganglionic stimuli long after the cells are seriously affected.

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REFERENCES.

Beznák, A. B. L. (1934). *J. Physiol.* **82**, 129.
Brown, G. L. (1934). *J. Physiol.* **81**, 228.
Brown, G. L. and Feldberg, W. (1936). *Ibid.* **86**, 10 *P.*
Cowan, S. L. (1934). *Proc. Roy. Soc.* B, **115**, 216.
Eccles, J. C. (1935 *a*). *J. Physiol.* **84**, 50 *P.*
Eccles, J. C. (1935 *b*). *Ibid.* **85**, 179.
Eccles, J. C. (1935 *c*). *Ibid.* **85**, 207.
Eccles, J. C. (1935 *d*). *Ibid.* **85**, 32 *P.*
Feldberg, W. and Gaddum, J. H. (1934). *Ibid.* **81**, 305.
Feldberg, W. and Vartiainen, A. (1934). *Ibid.* **83**, 103.
Gaddum, J. H. (1936). *Gefässerweiternde Stoffe der Gewebe.* Leipzig. Verlag Thieme, p. 148.
Hampel, C. W. (1935). *Amer. J. Physiol.* **111**, 611.
Howell, W. H. and Duke, W. W. (1908). *Ibid.* **21**, 51.
Vogt, M. (1936). *J. Physiol.* **86**, 258.